Macrophage Supply and Demand at the Core of the Necrotic Granuloma

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http://dx.doi.org/10.1016/j.chom.2015.06.014

Central necrosis of granulomas is linked to progression of major diseases, including tuberculosis and atherosclerosis. In this issue of *Cell Host & Microbe*, Pagán et al. (2015) reveal that necrotic granulomas develop when macrophage supply is insufficient. These findings suggest augmenting macrophage availability as a therapeutic strategy in tuberculosis.

With the rapid pace at which research on macrophages has advanced in the last several years, some major mysteries in macrophage biology remain scarcely explored and understood. One of these mysteries is understanding the functional properties of the macrophage-rich granuloma and interpreting the features that they display, ranging from multinucleated macrophages to necrotic cores at their center. In this issue, Antonio Pagán and colleagues in the laboratory of Lalita Ramakrishnan bring insight into the causal underpinnings behind the development of the necrotic core in tuberculosis (Pagán et al., 2015). They carry out a straightforward set of experiments in a zebrafish tuberculosis model, wherein central necrosis occurs in granulomas that develop following *Mycobacterium marinum* infection. The show that necrosis is a consequence of having too few newly arrived macrophages—fresh troops, if you will—to appropriately clear macrophages recruited at an earlier stage to the inflammatory battle, as these begin to die within the granuloma and thereby build necrotic debris. Through use of zebrafish bearing mutations that affect macrophage abundance and by adding other clever manipulations, they show that the development of the necrotic core coincides with the point in which macrophage demand within the granuloma outstrips the macrophage supply, arising most likely from monocyte precursors in blood. A major approach to modulating macrophage supply was deletion of the gene encoding colony stimulating factor 1 receptor (Csf-1r). As expected, loss of this receptor greatly reduces, but does not fully eliminate, the macrophage pool. The authors observed that in the early phases of granuloma formation, deficiency in Csf-1r had no impact on the accumulation of macrophages compared with control zebrafish. But there was a tipping point when the accumulation of macrophages failed to progress efficiently in the Csf-1r-deficient fish, and that point marked the emergence of the necrotic core. The authors went on deplete monocytes and macrophages to varying degrees or to promote their expansion by administering Csf-1. In each case, they could show that the nascent necrotic core corresponded to the point when macrophage supply failed to meet demand, or that the necrotic core could be prevented by expanding macrophages beyond a critical threshold. They concluded that the genesis and progression of the necrotic core is none other than a failure of macrophages to keep up with the need to remove macrophages dying in response to infection. This understanding is clinically significant because the necrotic core is rich in extracellular bacteria that most efficiently drive disease progression (Ramakrishnan, 2012).

The macrophage supply problem is likely ultimately linked to the supply of monocytes from the blood. Despite a growing appreciation for the role of resident macrophages as distinct from monocytes, monocytes are still the major source of macrophages in infectious and inflammatory contexts. The authors argued that while Csf-1r plays a role in baseline macrophage homeostasis, macrophage accumulation in granulomas of Csf-1r mutants was normal early on, so for a while the mutant could meet the demand for macrophages. However, the tipping point occurred at a later time when macrophage accumulation in the mutants failed to keep pace with the wild-type infected fish. The authors speculated that this failure corresponded to impaired emergency myelopoiesis in the mutant, a process that may be governed by Csf-1 signaling. While it would have been nice to see data on the number of circulating zebrafish monocytes before and during infection in mutant and wild-type fish, it is known that in mice loss of CSF-1R signaling minimally impacts the number of circulating classical monocytes—the ones recruited to extravascular tissues—but rather preferentially affects monocytes and macrophages at later stages (Lenzo et al., 2012). Thus, the idea that an early stage of granuloma formation might be normal is reasonable, as it would be built from the recruitment of the first wave of monocytes that may not be greatly reduced in the mutants. The subsequent collapse might relate to multiple issues, including impaired emergency myelopoiesis and impaired macrophage survival, proliferation, and differentiation. Since it seems clear that the blood is the ultimate source of these macrophages, one of the attractive implications of the study is that it suggests that the blood monocyte pool can be limiting in an inflammatory context. These findings give us more reason to revise a long-held view that monocyte numbers in the blood would generally be present in sufficient excess, if not in homeostasis, certainly during infection when numbers rise. This view likely arose from reasoning that the vast numbers of neutrophils present in the blood and turned over daily are mostly never called into action, and they are produced by the bone marrow in excess only to be ready to fight inflammation when it happens. However, in the last several years, a number of

observations cause us to question whether neutrophils and monocytes—all myeloid cells—actually circulate in blood to quantities that exceed demand. Supporting the idea that this is not the case are data that higher blood monocytes and neutrophils positively correlate with atherosclerotic plaques (Nahrendorf et al., 2010), where they are known to be recruited.

And herein lies a very interesting point for discussion that may extend the impact of the work of Pagán et al. (2015) beyond tuberculosis. Standing beside tuberculosis as a prevalent disease in which a "necrotic core" is a central and clinically significant feature is atherosclerosis, a leading killer in the developed world. The very reason that atherosclerosis causes death is that the contents of the necrotic core are highly prothrombotic; should the necrotic core rupture and spill its contents into the vasculature, occlusive thrombus results. Like tuberculosis, the necrotic core is comprised of dead macrophages, and it is surrounded by living macrophages that are thought to be on hand to clean up the necrotic debris. Parallels have been drawn between tuberculosis granulomas and atheroplaque (Seimon et al., 2010). Yet, opposite from the conclusion by Pagán et al. (2015), a major therapeutic concept in atherosclerosis is that stopping monocyte recruitment will be beneficial (Silvestre-Roig et al., 2014). However, while a cessation in monocyte recruitment may quell the pace of plaque expansion, the findings of Pagán et al. (2015) raise the possibility that an unintended consequence may be that the size of the necrotic core, at least relative to the plaque overall, would increase and plaques would be rendered less stable. Even though CSF-1 deficiency greatly reduces atherosclerosis development in mice, the possibility that CSF-1 might be in late stages protective of necrotic core advance has not been considered. Alternatively, tuberculosis and atherosclerosis may have parallels only to a point. In atherosclerosis, recruiting more macrophages may be insufficient to allow for engulfment of dying macrophages, as additional mechanisms are at play in preventing uptake of apoptotic and necrotic cells (Thorp and Tabas, 2009). Yet, the results of Pagán et al. (2015) might then be assembled into a hypothesis that protection from necrotic core-mediated death in atherosclerosis might be achieved not only from restoring dead cell removal but also making sure that plenty of "fresh troop" macrophages are assembled at the scene, either via recruitment or proliferation.

In summary, the straightforward experiments of Pagán et al. (2015) challenge us to reconsider what we thought we knew about the role of macrophages and CSF-1 in granulomas and their tendency to necrosis. Perhaps increasing the monocyte pool is a viable strategy to keep tuberculosis in check. Whether the lessons in tuberculosis apply to other scenarios of necrotic cores now emerges as an important question. The findings of Pagán et al. (2015) certainly deserve intense discussion in the tuberculosis and atherosclerosis communities and beyond.

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